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Research report

Effect of protein malnutrition on redox state of the hippocampus of rat

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Abstract

The protein malnutrition is a worldwide problem, affecting mainly newborns and children of developing countries. This deficiency reaches the brain in the most critical period of the development. Various consequences are related to this insult, such as memory disturbance, learning, and behavioral impairment. Protein content of the diet plays an important role on antioxidant mechanisms. This study observed the effects of protein malnutrition on rat hippocampus redox state. Wistar rats were separate in four groups, receiving different diets: first group with 25% casein, protein deficient group with 8% casein, and the same two groups supplemented with methionine (0.15%). Diets were isocaloric and were administered since the prenatal period up to the sacrifice. Rats were decapitated at 21 or 75 days old and hippocampus were isolated for measuring the lipoperoxidation by TBARS method, protein oxidative damage by carbonyl (DNPH) levels, and the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). There was significant alterations in the activities of the enzyme SOD, lipoperoxidation, and protein oxidation in hippocampus of 21 and 75 day-old rats fed with 25% of protein with methionine and the groups fed with low levels of protein (8%) both supplemented or not with methionine. Our data suggest that both the content of protein in the diet and the essential amino acid methionine may alter the antioxidant system and the redox state of the brain.

Theme: Development and regeneration
Topic: Nutritional and prenatal factors

Keywords: Protein malnutrition; Oxidative stress; Hippocampus; Methionine

1. Introduction

Protein malnutrition is a major problem in developing countries affecting mainly newborns and children during the most critical stage of their brain development. Protein deficiency can cause structural and functional deficits, affecting parameters as neuronal proliferation, migration, and myelination. Moreover, it has been shown that protein deficiency can affect several aspects of behavior and cognitive function, including learning and memory [24].

Protein deprivation during the most vulnerable period of the brain development may cause serious injuries affecting various biosynthetic process of the brain [15,23], phosphorylation of synaptic membrane proteins [32], neuronal connections, and neurotransmitter systems [24].

Several studies have shown that dietary protein is important for antioxidant mechanisms. It has been suggested that protein malnutrition may lead to an increase in oxidative damage by diminishing antioxidant defenses of the tissue [35]. Reactive oxygen species (ROS) are generated by biological systems during metabolism and can induce oxidative damage in organic molecules [17]. Although the organisms are able to protect themselves due

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to antioxidant machineries, an imbalance in the redox environment can be deleterious [17,22]. Importantly, ROS may also act as potential signaling molecules [6].

In the present work, we studied the effects of protein malnutrition on the redox state of hippocampus of rats. We have selected this structure because it is important for many plastic brain processes, such as learning and memory [4], which are strongly affected by malnutrition. We measured oxidative parameters and antioxidant enzyme activities in animals fed with a protein deficient (8%) diet, which were compared to animals fed with a 25% protein-containing diet. The protein diet consisted of casein supplemented with methionine 0.15% (final concentration), once casein is a poor source of this amino acid, which is an essential thiol donor and therefore an important sulfur-containing amino acid and methyl donor [22,25]. Considering the roles of methionine, we also investigated the effect of the methionine-containing diet on the redox state of the hippocampus of rats. Both the effect of the protein deficient diet (8%) and the supplementation of methionine on the diet were compared to a 25% protein diet.

2. Materials and methods

2.1. Reagents

Thiobarbituric acid, catalase, superoxide dismutase, dinitrophenylhidrazine, adrenaline, and hydrogen peroxide were purchased from Sigma, St. Louis, MO. Casein was purchased from Herzog, Porto Alegre, Brazil. L-methionine was purchased from Merck, Rio de Janeiro, Brazil. Mineral mixture and vitamin mixture were purchased from Roche, São Paulo, Brazil.

2.2. Animals and experimental model

Wistar rats from our breeding colony were used. The animals had free access to isocaloric diets (Table 1) containing 25% or 8% protein (casein), salts, and vitamins as recommended by the Association of Official Analytical Chemists [19] as previously described [29]. Four treatment groups were used at two different ages (21 and 75 days): first group was fed with a 25% protein diet (adopted as a normal, because resembled the commercial standard laboratory rat chows with 23–25% protein average); another group was fed with a 25% protein diet with methionine supplementation. Two other groups were fed with an 8% protein diet, with or without methionine supplementation. Prenatal and lactational malnutrition were induced in pups by restricting the mother's diet since the conception day until 21st postnatal day (weaning date), then the animals were fed with the same diet for either 21 or 75 days. Animals were killed by decapitation at the age of 21 or 75 days. The offspring size was adjusted the eight pups per mother on the first postpartum day. They were maintained at 22 °C, on a 12 h light/12 h dark cycle until the experimental age. The protocol concerning this research was used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

Table 1
Dietary content

Component	Casein diet	
	25%	8%
Casein (87% protein)[a]	28.75	9.25
Fat (soybean oil)	15.00	15.00
Carbohydrate (corn starch)	50.15	69.65
Salt mix[b]	4.00	4.00
Vitamin mix[c]	1.00	1.00
Non nutritive fiber	1.00	1.00

Percent (g/100 g diet) nutritional composition of the diets.
[a] Casein, purity 87% supplemented or not with 0.15% L-methionine, depending on the group.
[b] Mineral mixture mg/100 g of ration: NaCl, 557; KI, 3.2; KH_2PO_4, 1556; $MgSO_4$, 229; $CaCO_3$, 1526; $FeSO_4.7H_20$, 108; $MnSO_4.H_2O$, 16; $ZnSO_4.7H_2O$, 2.2; $CuSO_4.5H_2O$, 1.9; $CoCl_2.6H_2O$, 0.09.
[c] Vitamin mixture (from Roche, São Paulo, Brazil), mg/100 g of ration: Vitamin A, 4; Vitamin D, 0.5; Vitamin E, 10; Menadione, 0.5; Choline, 200; PABA, 10; Inositol, 10; Niacin, 4; Pantothenic acid, 4; Riboflavin, 0.8; Thiamin, 0.5; Pyridoxine, 0.5; Folic acid, 0.2; Biotin, 0.04; Vitamin B12, 0.003.

2.3. Thiobarbituric acid reactive species (TBARS)

As an index of lipid peroxidation, we used the formation of TBARS during an acid-heating reaction as previously described [11]. Briefly, the samples were mixed with 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% and then heated in a boiling water bath for 15 min. Malondialdeyde (MDA), an intermediate product of lipoperoxidation, was determined by the absorbance at 535 nm.

2.4. Measurement of protein carbonyls

The oxidative damage to proteins was measured by the quantification of carbonyl groups based on the reaction with dinitrophenylhidrazine (DNPH) as previously described [21]. Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH and the absorbance read at 370 nm.

2.5. Measurement of catalase (CAT) and superoxide dismutase (SOD) activities

Enzyme assays were performed in tissue extracts obtained as follows. To determine CAT activity (E.C. 1.11.1.6), each hippocampus was sonicated in 50 mM phosphate buffer (pH 7.0) and the resulting suspension was centrifuged at 3000 × *g* for 10 min. The supernatant was used for enzyme assay. CAT activity was measured by the